Staining of cells for flow cytometric analysis: extracellular antigens, directly conjugated antobodies

Materials:
FACSWash: PBS with 1% bovine serum albumin, 0.1% sodium azide
Antibodies

Methods:
1. Prepare cells:
   - Primary cells: Isolate cells to be stained, wash once in FACSWash, and resuspend in FACSWash at a concentration of approx 1-2 milliion/mL
   - Cell line: non-adherent: Spin cells out of media. Wash twice with FACSWash. Resuspend in FACSWash at above concentration.
   - Cell line: adherent: Trypsinize, scarape, or otherwise remove cells from flask, spin out of medi,a wash twice with FACSWash, resuspend at above concentration.
2. Aliqout cells – from 100,000 to 1 million - in 100uL FACSWash
3. Stain extracellular epitopes – add antibody at appropriate concentration (what manufacturer recommends for the number of cells you are staining, or what has been titrated to be the correct amount) directly to cells in the 100uL FACSWash. Vortex tube
4. Incubate on ice in dark for 30 minutes
5. Wash 1x with FACSWash
6. Bring to analyze on flow cytometer unfixed or fix in 1% paraformaldehyde and run within 3-4 days, depending on cells, fluorochromes, etc.

Important technical notes:
The only tubes that can be used on the flow cytometers are Falcon brand 12x75 tubes.
If you need some, ask the flow lab.

Some people fix in higher concentrations of PFA, which is fine, but if you plan to let them sit longer than 1 day, the lower conc. of 1% has worked better for us rather than 4%, which is fine if you run cells the next day.